THE EFFECT OF UREA ON IMMUNOLOGIC REACTIONS

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In a previous article [1] I reported that urea inhibited the action of rabbit complement. The present paper contains further results which show the effect of urea on human, guinea-pig, dog and hog complement, on normal dog antihuman hemolysins, and the effect of intravenous injections of urea and sodium chloride, respectively, on rabbit complement and the leukocyte count. These observations are paralleled with blood urea determinations.

The hemolytic system (antihuman) and the technic of studying the effect on complement in vitro was similar to that described.[1] The method used in determining the effect on complement in vivo of M/1 urea and M/1 NaCl solutions was essentially as follows:

The normal urea content per 100 c c of blood was determined several times in each rabbit. Immediately after the blood was drawn for the last determination 2 c c of M/1 urea were injected intravenously into one rabbit and 2 c c of M/1 NaCl into another. Repeated injections, varying in amount from 2 to 6 c c, complement titrations and blood counts were made approximately every hour. The quantitative determination of urea was essentially that given by Gradwohl [2] except that the ammonia was drawn over into a known amount of N/100 HCl and the amount of uncombined acid determined by direct titration, using methyl orange as an indicator. From this the amount of urea per 100 c c of blood was calculated.

It was found that complement of different animals differs markedly in its activity in the presence of urea. Human complement was inactive in the presence of 0.02 to 0.04 c c of M/1 urea, rabbit, hog and dog complements in the presence of 0.3, 0.1 and 0.2 c c, respectively, while guinea-pig complement did not seem to be materially affected. Normal dog lysins for human corpuscles as a rule failed to produce hemolysis in the presence of 0.3 to 0.4 c c M/1 urea. In my previous article,[1] I reported that urea did not prevent the union of amboceptor and corpuscles, and I have confirmed this result.

From a number of experiments it seems that the so-called "midpiece" of complement did not combine with the amboceptor-cell complex within the usual incubation period during which time the controls were completely laked.

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[1] Jour. Infect. Dis., 1917, 20, p. 185.

[2] Newer Methods of Blood and Urine Chemistry, 1917, p. 42.

As regards human complement, this effect is permanent, but in the case of rabbit, hog and dog complement, there is either a permanent interference or a marked slowing of the reaction. In the latter instance, if the tubes are placed in the icebox for 24 hours after the regular 30 minute incubation in the 37 C. water bath, hemolysis frequently results. This fixing or restraining effect did not occur with guinea-pig complement. There is no correlation between the amount of serum constituting a unit of complement and these results. The degree of the restraining effect seems to vary to some extent with the sample of urea. In all, 4 samples of chemically pure urea were used with quantitatively and qualitatively identical results for 3 samples and a slight quantitative difference for the fourth, which gives the slowing rather than the fixing effect observed for other than human complement which apparently underwent permanent "fixation."

It may be that the restraining effect slows down the reaction sufficiently to permit deterioration of complement. One has to consider the possibility of urea not being chemically pure. Mathews [3] states that small quantities of cyanamide may be present in urea solutions. In order to determine whether the urea I used contained cyanamide I made use of the qualitative test for cyanamide suggested by Caro, Schuck and Jacoby.[4] This is based on the observation that the silver salt is an amorphous yellow substance almost insoluble in dilute ammonia. I found this sufficiently sensitive to detect 1 part of cyanamide in 100,000 parts of dilute ammonia water. None of my samples of urea showed any evidence of containing cyanamide. Theoretically, the urea used in these experiments was chemically pure.

The blood urea determinations suggest that there is no correlation between normal urea content and the inhibitory effect of urea on complement. Human complement, which was most easily affected, contains very much less urea than that present in the other complements studied.

This work suggests fundamental differences in the complement of these animals, although this may be more apparent than real. It also indicates a new reason for the superiority of guinea-pig complement over other complements.

The results obtained by the intravenous injections of M/1 NaCl and M/1 urea are suggestive. The complement content compared with blood urea shows that following the injection of NaCl there was no accumulation of urea but instead an initial drop owing, probably, to its diuretic effect. The complement content either remained normal or was

[3] Physiol. Chemistry, 1916, p. 17.
[4] Cited by Franke, Cyanamid, 1913, p. 20.

increased. On the other hand, when M/1 urea was injected there was an initial drop in complement content associated with an increase in blood urea, followed by a rise associated with relatively low blood urea occurring apparently at the time of maximum activity on the part of the kidneys. Two hours later when the blood urea had risen from 47.3 to 60.2 mg. per 100 c c of blood there was a corresponding drop in complement content. During the next 4 hours the complement did not return to normal. The low complement content seemed to be associated with decreased activity on the part of the kidneys. This result suggests that a study of relation of renal function to complement content might be of value.

In reporting on the effect of any substance on the blood counts of rabbits, I am aware of a great normal variability which tends to lessen the significance of any comparative data. For this reason I would rather merely state results and not draw general conclusions. One injection of 2 c c of either M/1 urea or M/1 NaCl produced practically identical results, i. e., an initial leukopenia followed by a leukocytosis and then a return to normal within an hour or two. Occasionally there was a slight initial rise followed by a drop with a later rise to normal. Repeated injections of M/1 urea produced a leukopenia which persisted, as a rule, during the period of injection and from 1 to 4 hours after the last injection. This does not seem to hold, as a rule, for similar amounts of NaCl.

While Fosse [5] and others have carried out rather extensive work showing that urea is widely distributed in nature, very little seems to have been done to determine its exact physiologic and pharmacologic action. While it is a normal product, it is not present in normal persons as a rule in amounts much above 25 to 35 mg. per 100 c c of blood. In man, perhaps the most important precursor of urea is ammonia, while in some animals it is formed by oxidation and hydrolysis of uric acid. In man, the mechanism might be considered a protective one against the accumulation of an undue amount of ammonia. Perhaps urea normally has a definite physiologic function for man as cited by Mathews [3] for elasmobranch fishes. Von Furth [6] summarized the work of Heilner on the physiologic action of urea as follows: "Urea introduced subcutaneously has a stimulative effect on protein metabolism, thus suggesting the possibility of urea being a factor in the special mechanism regulative of the course of intracorporeal protein disintegration." Eyster [7] states that urea has a stimulative action on the heart.

[5] Ann. de l'Inst. Past., 1916, 30, p. 525.
[6] Chemistry of Metabolism, 1916, p. 499.
[7] Science, 1910, 31, p. 236.

These statements would indicate that relatively small doses of urea are apparently either of no effect or produce a stimulative one. As to large doses, Hewlett, Gilbert and Wickett [8] have shown that 100 gm. of urea given orally to man leads to a rapid rise of blood urea content associated with many of the symptoms of uremia.

The experiments I have reported are added evidence of the toxic effect of urea in the body when given in relatively large amounts and add to the significance of the results obtained in vitro. Clinically, in uremia, there has long been noted a lowered resistance to infection associated commonly with leukopenia.

SUMMARY AND CONCLUSIONS

Urea solutions restrain the union of complement with the amboceptor-cell complex.

The slowing effect varies for different complements ranging from permanent fixation for human complement to no appreciable effect for guinea-pig complement.

In test tube experiments it requires 10 times as much M/1 urea to inhibit rabbit and 7 times as much for hog complement and 10 times as much for dog complement as it takes for human complement.

Urea in NaCl solution in the concentrations stated does not directly lake red blood cells or interfere with the union of amboceptor and red blood cells.

The intravenous injection of 2 to 6 c c M/1 NaCl solution in rabbits gave a slight diuretic effect but no noticeable effect on complement.

Repeated injections of similar amounts of M/1 NaCl produced a slight initial leukopenia followed by leukocytosis in rabbits. One injection gave an initial leukopenia lasting for two hours, followed by a return to normal.

Repeated injections of M/1 urea solution was associated with a decrease in complement content.

The slight rise in complement two hours after the initial injection was associated with apparent maximum activity on the part of the kidneys.

Repeated injection of M/1 urea was associated with wide fluctuations in the leukocyte count, but on the whole produced noticeable leukopenia. One injection of 2 c c of M/1 urea gave an initial leukopenia followed by a marked leukocytosis, the blood count returning to normal within three hours and remaining normal.

[8] Arch. Int. Med., 1916, 18, p. 636.